Assessing toxicities of industrial effluents and 1,4-dioxane using sulphur-oxidising bacteria in a batch test

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Introduction

Discharge of effluents either from wastewater treatment plants or industrial sectors are primary sources of chemicals entering aquatic ecosystem (Gómez *et al*. 2001). Consequently, the contamination of soil and water resources (especially groundwater) with persistent organic pollutants is becoming an emerging concern (Han *et al*. 2001). 1,4-dioxane (1,4-D) is one such emerging organic pollutant, which is hydrophilic in nature and highly soluble in water (Duncan *et al*. 2004). 1,4-D is widely used as a solvent in a variety of commercial and industrial applications such as for manufacturing plastics, pesticides, inks, dyes, paints, cosmetics, deodorants, rubber, oils and resins (Son *et al*. 2006; Otto & Nagaraja 2007; Sei *et al*. 2010). The contamination of groundwater with 1,4-D has become a major threat to the existence of living organisms in the aquatic environment as 1,4-D results in both acute and chronic toxicity (Stickney *et al*. 2003). 1,4-D is highly mobile which may migrate rapidly in groundwater, ahead of other pollutants, and does not volatilise readily from surface water bodies (Duncan *et al*. 2004; EPA 2006). It is also classified as a probable human carcinogen (Beckett & Hua 2000; Stickney *et al*. 2003). Depending on the extent and duration of exposure to 1,4-D, both humans and animals can experience several adverse health conditions such as irritation of eyes, nose, skin and the respiratory tract, and can even cause lung, liver and kidney damage (Johnstone 1959; Derosa *et al*. 1996; EPA 2009). Therefore, the rapid and continuous monitoring of industrial effluent is of great importance to ensure that they will not cause adverse effects on the aquatic environment.

The assessment of industrial effluents based on the biological effects of their discharge to the aquatic ecosystem is a matter of increasing concern (Tonkes *et al*. 1999; Farre & Barcelo 2003; Kim *et al*. 2008). In many countries, local governments still rely on physicochemical characteristics of effluents to regulate and manage effluents produced by industrial sectors (Liu *et al*. 2002; Ra *et al*. 2007; Jo *et al*. 2008). Physicochemical parameters are insufficient to characterise effluents and to predict the potential harmful effects of chemicals on the aquatic environment (Sahu *et al*. 2008). Chemical analysis alone has difficulty assessing complex mixtures of substances in industrial effluents (Eltzov *et al*. 2009). Hence, the toxic effects of these complex mixtures can only be detected using biological toxicity tests (Chen *et al*. 1999; Kohler *et al*. 2006).

Biological tests respond to the total effect of chemical activity (Blaise *et al*. 1988). Hence, bioassays have been used extensively for the assessment of toxic effects of complex mixtures in industrial effluents (Tanaka *et al*. 2002; Schmitt *et al*. 2005). Numerous methods have been developed and applied to living organisms such as fish, algae, daphnids, plant cells, animal cells and several bac terial species to assess the toxicity of wastewater samples (Sponza 2003; Girotti *et al*. 2008; Palma *et al*. 2009). Microbial bioassays have many advantages for the detection of toxic substances because they are inexpensive, readily available, easily reproducible and produce a quick response towards toxic substances (Tzoris *et al*. 2005; Girotti *et al*. 2008).

Recently, a novel biosensor for detecting toxicity in water using sulphur-oxidizing bacteria (SOB) has been developed (Oh *et al*. 2011). SOB are chemoautotrophic bacteria, which have the ability to use reduced sulphur compounds as an energy source to produce sulphuric acid (H_2SO_4) under aerobic conditions. Most of SOB can oxidise elemental sulphur (S°) according to Equation (1) (Madigan *et al*. 2009):

Please refer to the full text

S° is extremely insoluble and SOB must attach to the sulphur particles. The production of H_2SO_4 ultimately lowers the pH of the medium, and the production of sulphate ions (SO_4^{2-}) increases the electrical conductivity (EC) of the medium (Madigan *et al*. 2009). In a medium, EC is proportional to the concentration and type of ions and is a measurement of the ability of the medium to carry an electric current (Oh *et al*. 2011). If toxic chemicals are present, the activity of SOB will be inhibited and results in an increase in pH and a decrease in EC (Hassan *et al*. 2010; Van Ginkel *et al*. 2010).

This paper presents a preliminary study using SOB in batch tests to assess and evaluate the inhibition of effluent samples collected from nine anonymous industries, mainly discharging 1,4-D as effluents, by measuring the EC and pH over 12 h. The main objective of this study was to evaluate the employment of SOB to assess the inhibition of industrial effluents.

Materials and methods

Culture and medium

Aerobic return activated sludge (1 mL) was used as the inoculum to a sulphur master-culture reactor (SMCR) containing S°. The sludge was taken from the Chuncheon Wastewater Treatment plant in Chuncheon City, Kangwon-do, Republic of Korea. Synthetic stream water was prepared by diluting the following nutrient mineral buffer (NMB) solution 100 times: $NaHCO_3$ (3.13 g/L), NH_4Cl (0.31 g/L), $NaH_2PO_4.H_2O$ (0.75 g/L), KCl (0.13 g/L), NaH_2PO_4 (4.22 g/L), Na_2HPO_4 (2.75 g/L). Trace metal and vitamin solutions, previously described by Lovley and Phillips (1988), were also diluted 100 times and added to the synthetic water. The pH, EC and alkalinity of the synthetic stream water were 7 _ 0.2, 0.12 mS/cm, and 45 mg/L as $CaCO_3$. However, the pH, EC and alkalinity values were relatively high in all industrial effluent.

Reactor construction and batch experiments

S° (600 g, < 1 mm in diameter) was placed in a 1-L beaker (Diamond, Korea) and filled with 600 mL diluted NMB solution. Air was introduced to the SMCR using a stone diffuser with a flow rate of 150–250 mL/min. The SMCR was inoculated with sludge and incubated at 30°C in a semi-continuous mode. From the SMCR, 200 mL was wasted and refilled with 200 mL diluted NMB solution at 4–5-day intervals. The initial pH and EC of the SMCR were 7.31 and 0.07 mS/cm, respectively (Fig. 1). The SMCR was operated for 30 days to reach steady-state conditions (i.e. pH = 1 and EC = 18 mS/cm) and then used for the SOB batch test.

Effluents samples were collected in 10-L polyethylene jars from nine anonymous industries: A, B, C, D, E, F, G, H and I, located in the vicinity of Gumi City, Gyeongsangbukdo Province, Republic of Korea. Collected effluent samples were transported on ice and stored at 4°C prior to the experiments. The experimental concentrations were 100, 85, 50, 25, 10, 5 and 0.5% of the industrial effluents, and the dilution water was prepared with the same formulation used for the synthetic stream water. Experiments were carried out in 100 mL media bottles (Horex, Germany) that contained 30 mL of mixed liquor (effluent and dilution water). 15 g of sulphur particles from the SMCR were used as the test micro-organisms for monitoring inhibition of the effluents. The test bottles were capped with aluminium foil to prevent evaporation, and the tests were conducted for 12 h at 30°C and agitated at 150 rpm for air diffusion (SI-600R, Korea).

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